THE FIBRILLAR SYSTEMS OF CILIATES AS REVEALED BY THE ELECTRON MICROSCOPE. II. TETRAHYMENA[1]

CHARLES B. METZ[2] AND JANE A. WESTFALL

Department of Zoology, University of North Carolina, Chapel Hill, North Carolina

Recently the fibrillar systems of ciliates have attracted the attention of several investigators. This revival of interest has resulted in large measure from an appreciation of the morphogenetic properties of these systems. These properties appear to include self-duplication, genetic continuity, pluripotency and a role in the establishment of the polarity and fine morphology of ciliates (Fauré-Fremiet, 1948a; Lwoff, 1950; Weisz, 1951). Unfortunately, continued progress of these studies has been limited by an inadequate understanding of the micro-structure of ciliate fibrillar systems. However, a clearer knowledge of these structures has recently been obtained from an electron microscope study of *Paramecium* (Metz, Pitelka and Westfall, 1953). The present investigation was undertaken to extend this study to other material.

Tetrahymena was selected for this second effort not only because of the wide popularity of this organism as a laboratory animal but also because it can be cultured readily in the absence of other organisms; its fibrillar morphology has been studied exhaustively within the limits imposed by light optics (most recently by Corliss, 1952a, 1953); its morphogenesis at fission has been examined in detail (Chatton, Lwoff, Lwoff and Monod, 1931; Furgason, 1940); and finally its locomotion appears to involve an acetylcholine-acetylcholinesterase system (Seaman, 1951; Seaman and Houlihan, 1951).

The pellicle and oral anatomy of *Tetrahymena* are found to differ greatly from that of *Paramecium* but the kinety (silver line or neuromotor) systems of the two organisms are basically similar in structure and organization. The kineties of *Tetrahymena*, like those of *Paramecium*, are compound structures composed of discrete units. Each unit consists of a cilium, a kinetosome (ciliary basal body) and a short, tapering, kinetodesmal fibril with periodic structure. As reported briefly elsewhere (Metz, 1953), these units are associated by overlapping of the individual kinetodesmal fibrils to form the kinetodesma or silver line fiber of the light microscopist.

Material and Methods

Tetrahymena gelcii,[3] strain W, was used exclusively in this investigation and the writers are indebted to Dr. G. W. Kidder for the initial culture of this organism. All subsequent cultures were grown in 2% proteose peptone in the absence of other organisms. Three- to seven-day old cultures served as starting material for electron

[1] This investigation was aided by a grant from the American Cancer Society.

[2] Present address: Department of Zoology, Florida State University, Tallahassee, Florida.

[3] Following an exhaustive study Corliss (1952b) concludes that the specific name *pyriformis* should have priority over *gelcii* (Furgason, 1940).

microscope examination. With few exceptions these rich cultures revealed no dividing animals when examined with light optics.

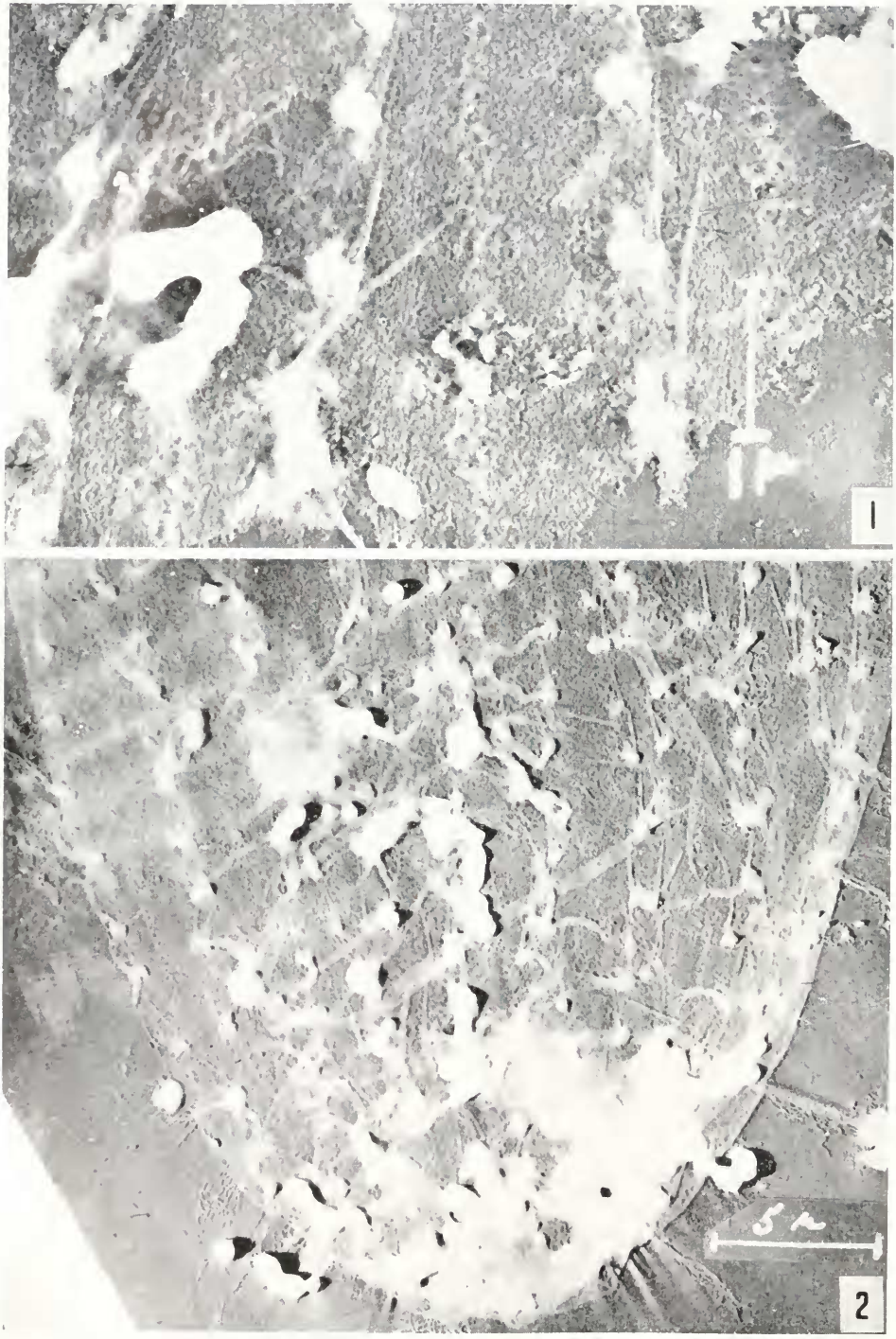
Preparation of the material for electron microscopy

The preparation procedure employed in the *Paramecium* study (Metz, Pitelka and Westfall, 1953) was transferred to *Tetrahymena* with little modification. It consisted of the following steps: (1) fixation, (2) distilled water washing to remove fixative, (3) sonic dissection (9 Kc magnetostriction oscillator, model S-102A, Raytheon Corp.), (4) fractional centrifugation to isolate and concentrate pellicular fragments, (5) mounting (air drying specimens to collodion-coated electron microscope screens) and (6) shadow casting with chromium. The critical dissection, concentration and mounting operations (steps 3, 4 and 5) were followed with phase optics.

Formalin and osmium tetroxide vapor were employed as fixatives. Formalin fixation was achieved by mixing equal volumes of untreated *Tetrahymena* culture and diluted formalin (commercial formalin diluted to 1 15 with distilled water giving a final formalin concentration of 3.3% in the mixture). The mixture was allowed to stand for one to several hours and finally processed as described above. To obtain osmium tetroxide fixation, one to several ml. of *Tetrahymena* culture (with or without previous concentration by centrifugation) were poured into a syracuse or petri dish and exposed to osmium tetroxide vapor in a closed vessel. Following fixation the material was processed as outlined above. The osmium tetroxide material was examined most extensively. Although this method of preparation sometimes gave beautiful preparations, it did not do so consistently. In many cases the fibrillar apparatus appeared to be coated with precipitated cytoplasm and mitochondria when examined with electron optics. Indeed the kineties of *Tetrahymena* appeared to serve as preferential sites for such precipitation and in a number of preparations the primary kinety fibrils were completely obscured by such material. This difficulty was encountered in other forms, notably *Blepharisma* and *Stentor*, in preliminary examinations. This variable behavior may have an important bearing upon the results obtained with the silver impregnation technique (*e.g.*, von Gelei, 1935; Corliss, 1952a). With limited access to the electron microscope it was not possible to standardize the procedure to the point where consistently favorable results were obtained. However, fixation appears to be the critical operation. Prolonged treatment with osmium tetroxide vapor is to be avoided. Just sufficient exposure to kill the organisms, followed by judicious sonic treatment (phase microscope observations at 30-second to one-minute intervals), gave the most satisfactory preparations. Indeed the best specimens were fixed so lightly that the cilia frayed out (Figs. 1, 2) and macronuclear membranes sometimes broke down revealing interesting internal structure. Evidently some fixation is necessary, for all attempts to obtain pellicular fragments by sonic treatment of living animals produced only breis.

Electron microscope examination

The material was examined with an RCA model EMU electron microscope using standard procedures. However, one unusual detail of procedure must be emphasized. This concerns the orientation of the specimen in the electron microscope.



FIGURES 1–2.

The specimens are fragments of the protozoan surface, they are asymmetrical (three axes of asymmetry, *e.g.* Figs. 1, 6) and their relations to the morphology of the intact animal must be interpreted in terms of this asymmetry. The specimen screen may be mounted in the electron microscope so that the specimen side of the screen faces toward or away from the photographic plate of the instrument and either arrangement is acceptable practice. However, a reversal of symmetry will result in changing from one situation to the other. When the specimen surface of the screen faces away from the plate, normal symmetry relations obtain on the plate; when the specimen surface faces toward the plate, normal symmetry is reversed and a mirror image results. This problem is seldom critical in electron microscopy (Hillier, personal communication) but its importance cannot be over-emphasized in the present instance.[4] It follows that all subsequent photographic manipulations (reversal or "second negative" and final printing) must be performed with equal attention to symmetry.

The screens were always mounted specimen side down in the electron microscope specimen screen holder (facing the photographic plate). Proper orientation was facilitated by using Athene screens (Smethurst High-Light Limited) which have easily recognizable differences in their surfaces.

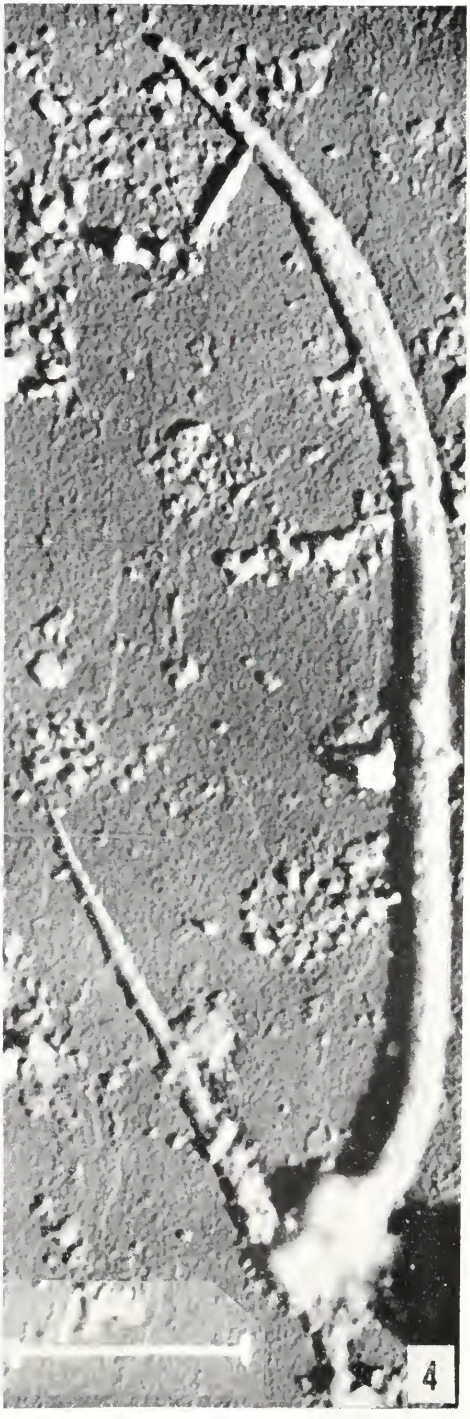
Limitations of the method

The rather drastic methods of preparation employed introduce certain hazards and limitations to the study. These include the following: (1) artifacts may be introduced by fixation, sonication and drying; (2) observations are usually made on fragments of many organisms selected at random, making it difficult to reconstruct the morphology of a single individual; (3) the sonic dissection method involves removal of parts of the organism under conditions that preclude direct observation. These limitations have been discussed and evaluated in the previous study (Metz, Pitelka and Westfall, 1953). They apply with nearly equal force in the present instance. However, *Tetrahymena* does possess two striking advantages over *Paramecium*. First, when grown in the absence of other organisms, all material observed with the electron microscope may be considered to be a part of the organism or a byproduct of it, if suitable precautions are taken to prevent organic and other contamination during preparation. Second, *Tetrahymena* is of such a size that large, easily recognizable fragments or whole, "eviscerated" animals can be examined with the electron microscope (*e.g.,* Fig. 2). Both of these advantages were exploited in this investigation.

[4] These precautions were followed in the Paramecium study although they were not stressed in that paper.

FIGURE 1. A fragment of pellicle viewed from the inner surface. Segments of three kineties are shown. Each of these consists of several kinety units (3, 4 and 5 units in the right, middle and left kineties, respectively). Each kinetodesmal fibril is seen to arise from a kinetosome and overlap the next unit. Bases of the cilia pass through ring-shaped thickenings in the pellicle. The striated area of the pellicle is seen immediately to the right of the kineties. The chains of ring-shaped thickenings in the pellicle are evident midway between the kineties. The granular area of the pellicle is located to the right of the pellicular chains. Part of a frayed cilium is visible through the pellicle (upper left). Osmium tetroxide fixation.

FIGURE 2. Posterior end of an "eviscerated" animal. The individual kinetodesmal fibrils are directed toward the anterior end of the animal. Frayed cilia may be seen on the margins of the preparation. Osmium tetroxide fixation.



FIGURES 3–4.

Results

When properly prepared by osmium tetroxide fixation the material contains fragments of pellicle of various sizes (Figs. 1–3), the isolated oral apparatus (Figs. 5, 6) and formed bodies of the cytoplasm (mitochondria, macronuclei). The units of kinety structure generally remain attached to the pellicular fragments in osmium tetroxide-fixed material but they may be obtained in isolated form (Fig. 4) from formalin-fixed animals. Since the electron photomicrographs show interesting details of structure in the pellicle, kinety system and oral apparatus, these will be considered separately.

A. Structure of the pellicle

The pellicle is a thin membrane with three structurally distinct regions. These are most evident in Figure 1. This figure shows that the inner surface of the pellicle consists of (1) a "background area" of rather uniform granular appearance, (2) a "chain" of ringlike thickenings parallel to and midway between the kineties and (3) a finely striated area immediately to the right of each kinety.

Background area

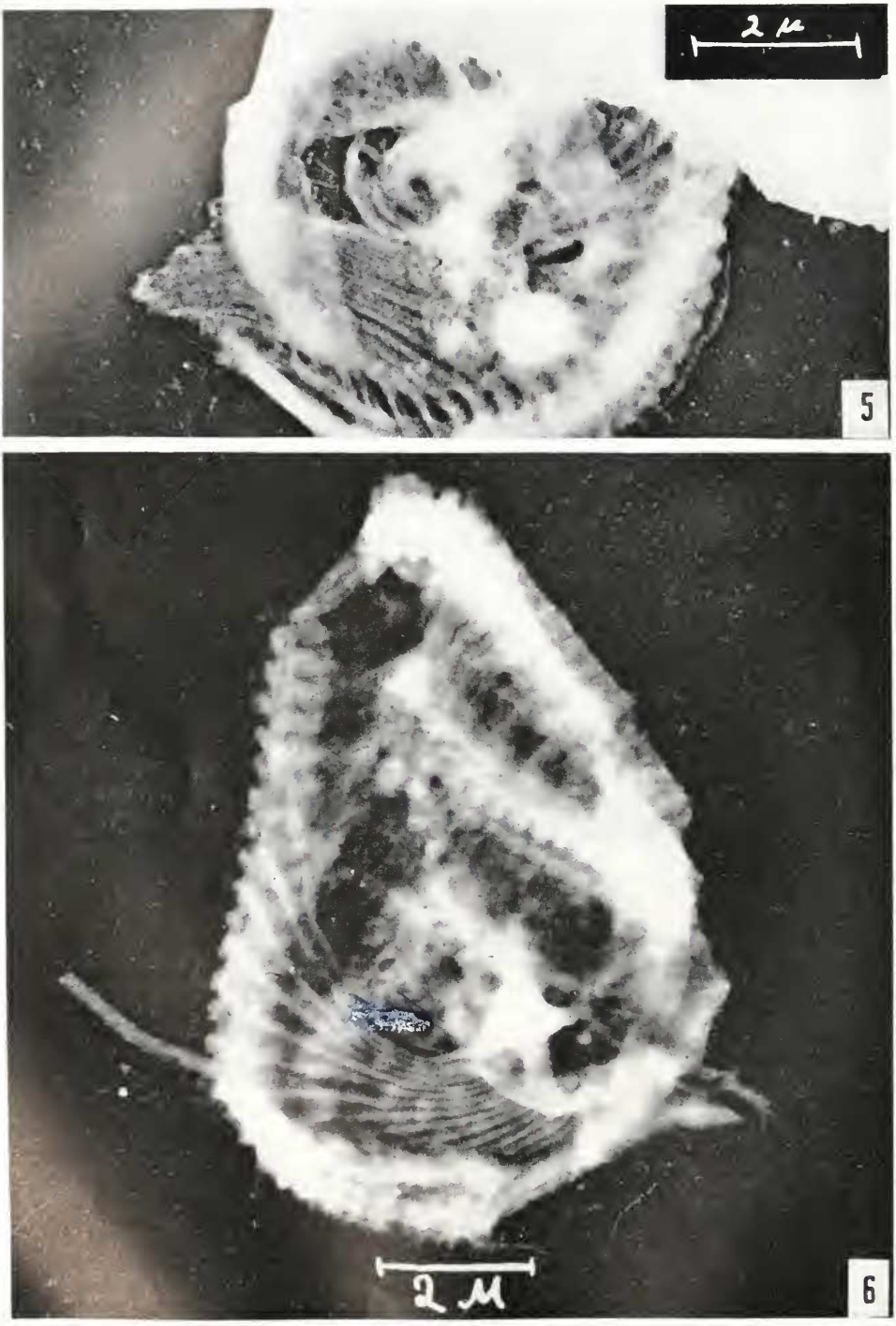
This region warrants little comment. The granular appearance of this material may be ascribed to macromolecular components of the inner surface of the membrane or to sub-pellicular protoplasmic components which have remained attached to the membrane proper. Study of this region at high magnifications and with special techniques might reveal further interesting details, possibly comparable to those of the erythrocyte membrane (Hillier and Hoffman, 1953).

Chains of ring-like thickenings

The chains of ring-like thickenings are the most striking structural feature of the pellicle. Two systems of such chains appear to be present in *Tetrahymena*. The first and more prominent of these occurs with great regularity and consists of chains located midway between, and parallel to, the kineties. The second, less evident system parallels both the first system and the kineties and is located at the level of the kineties. This second system is found in many but not all preparations but it may occur generally, for this region of the pellicle is frequently obscured to a greater or less extent by the kinetosomes, kinetodesmal fibrils and cilia. The

Figure 3. "Simulated cross section." The pellicular fragment has folded upon itself. The fold forms the left margin of the specimen, the outer surfaces of the pellicle are opposed, with the frayed cilium from the central kinety unit sandwiched between these surfaces. The fold is oblique to the axis of the animal. Therefore the three prominent kinety units belong to three different kineties. The chains of ring-shaped thickenings run diagonally from lower right to upper left in the upper layer of the pellicle. They pass around the fold to the lower layer of pellicle and proceed from lower left to upper right, thereby forming an angle of approximately 90° with the parent row in the upper layer. The two systems of ring-shaped chains may be seen in this preparation. One of these is located at the level of the kineties, the other runs midway between kineties. Osmium tetroxide fixation.

Figure 4. The isolated unit of kinety structure. The long cilium consists of a narrow terminal portion, the main shaft with indications of longitudinal fibrils and the basal bulb. This bulb is to be distinguished from the kinetosome situated just below the bulb (compare with Fig. 3). The kinetodesmal fibrils which arise from the kinetosome again show periodicity. Formalin fixation.



FIGURES 5–6.

first system but not the second is evident in Figures 1 and 2. Both systems are present in Figure 3 (see caption to Fig. 3 for details).

Although the chains of ring-shaped thickenings occur with great regularity, their form is somewhat variable. This variability may be ascribed in part to variations in fixation. The rings are usually more prominent following prolonged fixation (osmium tetroxide) and their centers frequently appear more transparent in such preparations. Other variations can not be ascribed to fixation and may result in part from uneven stretching of these thin membranes during drying. In its simplest form the chain is a single even row of closely spaced rings (Fig. 2) but this construction, while not uncommon, is sometimes modified so that the chains consist of bands two or more rings wide (Fig. 1). In other preparations the chains may branch or the rings may be widely scattered in the granular area of the pellicle. However, these last variations are exceptional.

The striated area

The delicate striated areas are apparent only in favorable preparations but in these they occur regularly and are constant in position and form (Fig. 1). These areas are situated immediately to the right of each kinety.[5] Each consists of a narrow (0.25 μ wide) band of material which runs parallel to the adjacent kinety and to the long axis of the animal. The characteristic feature of these bands is the collection of fine, closely spaced ridges (fibers?) that run lengthwise along the bands. The origin, composition and function of these bands are unknown. They are assumed to be part of the pellicle proper because of their constancy of occurrence and form. However, the possibility that they consist of sub-pellicular material adhering to the pellicle has not been excluded.

B. The kinety system

It will be recalled (Metz, Pitelka and Westfall, 1953) that the kineties of *Paramecium* are compound structures. They are built up of discrete units. Each of these units consists of a cilium, a kinestosome (ciliary basal body) and a relatively short, tapering, kinetodesmal fibril. These units are associated by their kinetodesmal fibrils. The fibrils overlap in shingle-like fashion to form a bundle,

[5] "Right" as employed here refers to the animal's right. When the pellicle is viewed from within and the figure is oriented with the anterior end of the animal upward (Fig. 1), then the animal's right will correspond to the observer's right. When the fragment is viewed from the outside, with the anterior end upward, the animal's right will be the observer's left.

FIGURE 5. The posterior part of the oral apparatus viewed from its inner surface. The "fan" of parallel fibers is clearly seen to pass to the left and beyond the margin of the oral apparatus from the undulating membrane (membranelle 1). At their origins the fibers appear to be in pairs or to have double origins in the lower part of the figure. Traces of the complex systems of fibers which interconnect the membranelles are visible in the upper part of the figure. Osmium tetroxide fixation.

FIGURE 6. The oral apparatus viewed from the outer surface. The base of the undulating or first membranelle passes along the right side (observer's left) of the preparation. A portion of one cilium remains attached to the base of this membranelle. The base of the second membranelle is located on the anterior left margin of the preparation. The third and fourth membranelle bases are located more posteriorly. The fan of fibers from the first membranelle passes to the left and under the left margin of the preparation. These fibers presumably pass down the wall of the cytopharynx in the intact animal. See text for further details. Osmium tetroxide fixation.

the kinetodesma (neuromotor or silver line fiber) of earlier light and electron microscope studies (Bretschneider, 1950; Sedar, 1952). The kineties of *Tetrahymena* are similar in structure to those of *Paramecium* in all important essentials.

The unit of kinety structure

Each kinety of *Tetrahymena* is composed of discrete units. One such unit is shown in Figure 4. This isolated structure is composed of a cilium, a kinetosome and a kinetodesmal fibril. The cilium and kinetodesmal fibril form an angle of approximately ninety degrees as they arise from the kinetosome.

The *cilium* of *Tetrahymena* has the usual structure since it appears to consist of an outer limiting membrane (Fig. 4) enclosing a number of longitudinally arranged fibers. With mild fixation the membrane breaks down and the fibers fray out (Figs. 1, 2, 3). In the intact cilium (Fig. 4) three regions may be distinguished. These include 1) the main shaft, 2) a distal, narrow portion constituting $^1/_6$th of the cilium and 3) a proximal bulb. A similar narrow tip has been described in *Paramecium* (Metz, 1954) but the proximal bulb is evidently unique. This bulb is located at the base of the cilium as the latter structure passes through the pellicle. The proximal ciliary bulb should not be confused with the kinetosome. These two structures are separated by a constriction at the level of the pellicle. The relative positions of the ciliary bulb, pellicle and kinetosome may be determined by comparing Figures 3 and 4. Figure 3 shows a simulated cross section—actually a folded pellicular fragment with the outer pellicular surfaces opposed (see caption to Fig. 3 for details). One kinety unit is located on the margin of the fold and the pellicle is seen to pass between the proximal bulb of the cilium and the kinetosome. The ciliary bulb may be an artifact of preparation. However, its presence in both formalin and osmium tetroxide preparations does not favor this possibility.

The *kinetosome* appears to be a double structure (over-all dimensions 0.2 $\times$ 0.3 μ) consisting of two bodies separated by a constriction perpendicular to the surface in the transverse plane of the animal. One of these bodies, the primary one, appears to be slightly larger than the other and gives origin to both the cilium and the kinetodesmal fibril. The second body forms an appendage to the first, 180° from the site of origin of the kinetodesmal fibril. These relations are evident in Figure 3.

The *kinetodesmal fibrils* arise from the kinetosomes and run just below and parallel to the pellicle (Figs. 1, 2, 3). These structures gradually taper to a fine point from a base diameter of approximately 0.12 μ (calculated from Figs. 3 and 4; it is assumed that the specimens have suffered no appreciable flattening since they cast well proportioned shadows). Calculations from several preparations indicate a length of 1.5 to 2 microns for the fibrils. The average length of fibrils in Figure 1 is 1.5 μ; lengths in Figures 3 and 4 are 2.1 microns. The kinetodesmal fibrils show an interesting periodicity in favorable shadow-cast preparations (especially clear in Fig. 3). This periodic organization appears as a series of transverse ridges separated by constrictions in the preparations so far examined, but further study may resolve these into spiral ridges similar to those of the *Paramecium* kinetodesmal fibrils (Metz, Pitelka and Westfall, 1953).

Association of the units to form kineties

In *Tetrahymena* as in *Paramecium*, the units of kinety structure do not appear to exist as unrelated, randomly arranged, isolates. They are organized to form

larger structural (and probably functional) units, the kineties. The units are arranged in longitudinal rows with the kinetosomes and cilia spaced at regular intervals (Figs. 1 and 2). The individual kinetodesmal fibrils are directed along these longitudinal rows and since they are longer than the distance between kinetosomes, they overlap in shingle-like fashion (Fig. 1). This overlap associates one kinety unit with the next adjacent unit. The series of overlapping fibrils constitutes the kinetodesma of the light microscopist. This construction is essentially the same as that found in *Paramecium*. In *Tetrahymena*, however, the kinetodesmal fibrils are so short (less than two interciliary distances) that true bundles of fibrils are never formed. A cross section of a kinety at any level should not show segments of more than two fibrils. In *Paramecium* the maximum number appears to be five.

In life the association of the fibrils in the region of overlap is presumably an intimate one and it appears to be so in some electron photomicrographs (Fig. 2), but in many preparations (Figs. 1, 3) the fibrils are widely separated at the region of overlap. This may be ascribed to the violent action of the sonic treatment, surface tension forces during drying and the relatively small area of contact between the fibrils. This failure to observe intimate association in most cases serves to emphasize the apparent absence of any sheath or cementing material binding the fibrils together. Indeed, no information is yet available concerning the details of this association. It is one of the more interesting problems arising from the study.

Polarity

Examination of the figures reveals that the kinetodesmal fibrils of the unit structures all extend in the same direction from their kinetosomes. They are polarized. Therefore, it becomes a matter of some interest to establish the direction of this polarity; to determine whether the fibrils extend toward the anterior or posterior end of the animal. Three independent observations bear upon this problem and they show that the fibrils pass toward the anterior end of the animal. These observations are the following: 1) The kinetodesmal fibrils pass to one side of the next adjacent kinetosome (Fig. 1). This arrangement is regular and consistent in all electron photomicrographs except for an occasional fibril. These exceptional cases may be ascribed to alterations of the pattern during preparation. Indeed it is astonishing that the pattern is so regular considering the violent nature of the treatment. This pattern is believed to be the basis for the "rule of desmodexy" (Chatton and Lwoff, 1935; Lwoff, 1950). This rule states that kinetosomes always lie to the left (animal's left) of the kinetodesma. When the photomicrographs are oriented according to desmodexy, the fibrils are found to pass forward, *i.e.*, anteriorly. This relation is illustrated in Figure 1. Since this figure shows the inner surface of the pellicular fragment, the observer's right and left correspond to the animal's right and left. 2) The isolated oral apparatus is highly asymmetrical (Fig. 6) and is easily oriented (see next section) with respect to the anterior-posterior axis of the animal. Occasionally the oral apparatus is isolated with pieces of adjacent pellicle attached and the orientation of the kinetodesmal fibrils attached to such pellicle can be established with respect to the anterior-posterior axis of the neighboring oral apparatus. Nine cases of this sort were examined. In eight of these the fibrils were directed anteriorly. In the 9th the fibrils were directed posteriorly but this may not be a valid case for it is possible

that this figure represents an isolated oral apparatus lying over a fragment of unrelated pellicle. 3) When subjected to mild sonic treatment large segments of individual animals may be obtained. In favorable preparations these are largely free of internal protoplasm. Such "eviscerated" animals may retain the original form of the organism. The posterior end of such a preparation is illustrated in Figure 2. The fact that there are two layers of pellicle, one over the other, renders it difficult to follow some of the kineties and to determine which kinety belongs to which layer, but the figure clearly shows that the individual kinetodesmal fibrils pass anteriorly from their kinetosomes.

C. Oral anatomy

The preparations obtained by sonic dissection contain large numbers of oral structures in various degrees of isolation (see previous section under polarity). Representative examples are shown in Figures 5 and 6. With the exception of the oral cilia these preparations appear to consist of substantially the entire oral apparatus including the four "membranelles" and their interconnecting fiber systems.

The orientation of these isolates is readily established. This may be done by reference to the abundant figures in the literature from light microscope studies, by reversing argument number 2 presented above in the section on polarity, by examining large fragments (argument No. 3 in the above section on polarity) and by phase microscope examination of fixed material preliminary to sonic treatment. Applying these criteria the specimen illustrated in Figure 6 is found to be an outside view of the oral complex and the foundations of three of the four "membranelles" are readily identified. The right (animal's right, observer's left) and about half the posterior margin of the oral complex consist of the first of these, the so called "undulating membrane." A portion of one component cilium remains attached to the foundation of this membrane. The second "membranelle" is readily identified on the left (animal's left, observer's right) anterior margin and the third is located immediately posterior and nearly parallel to the second. The fourth "membranelle" is almost certainly situated in the electron-opaque material posterior to the third "membranelle," where a number of kinetosomes appear to be located. However, this region has not been observed clearly in the outside views of the oral apparatus and it is equally obscure in inside views. These complexes and their interconnecting fiber systems invite the detailed descriptions given below.

Membranelle No. 1 (undulating membrane)

The foundations of the membranelles are relatively dense and reveal less detail than might be desired. The first membranelle is no exception. It appears to consist of a marginal row of cilium bases, presumably kinetosomes. Segmentally arranged masses of material, 0.8 μ long, extend "medially" (presumably toward the interior of the animal) from these. They appear to correspond in number, but not necessarily in position to the ciliary bases and are readily identified in the anterior portion of the membranelle (Fig. 6). In some figures (Fig. 5) these masses appear to be broken up in a fashion to suggest at least three parallel rows of cilium bases. However, light microscope studies (*e.g.*, Furgason, 1940) show only one row of cilia in this membrane and electron microscope figures of specimens with intact cilia support this view. A suggestion of a longitudinal fiber (or series of

short overlapping fibers?) is visible just medial to the cilium bases in the anterior end of the membranelle foundation (Fig. 6).

Although these details of structure have their interest, the most striking feature of the membranelle is the fan-shaped series of fibers that extend from it. In the figures this fan extends toward the left of the oral apparatus, under the left posterior margin and beyond. This is clearly shown in Figures 5 and 6. The fans of fibers illustrated in these figures are broken off relatively close to their origins. In some other figures, however, they appear to be relatively intact. In these they are found to extend at least 13 microns beyond the left margin of the oral apparatus. The fan of fibers tapers as it extends; whether this taper results from decrease in fiber number or from reduction in fiber diameter (or both) has not been determined, but if there is no reduction in number, the longest fibers must extend for approximately 20 microns (nearly half the length of the animal) from their bases. Presumably these fibers extend down or close to the right side of the gullet in the intact animal, but where they end has not been established.

The relation of these fibers to the kinetosomes of the membranelle cilia presents an interesting challenge to the microanatomist. The individual fibers of the fan arise at or near the kinetosomes of the membranelle. However, there appear to be fewer fibers than kinetosomes (16 and 28, respectively, in Fig. 6). No fibers are seen to extend to the first three or four kinetosomes in Figure 6, whereas more posteriorly a fiber seems to extend to every second kinetosome. The function of these fibers and their relation to other organelles present interesting problems for future study. The fact that some of the fibers appear double in Figure 5 enhances this interest.

Membranelles II, III and IV

The second and third membranelles each appear to consist of at least three rows of ciliary bases or kinetosomes. This is evident only for membranelle III in Figure 6. Figures not reproduced here show a similar condition in membranelle II. The region of membranelle IV has such high electron-scattering power that little detail is revealed in any of the photomicrographs so far obtained. A single row of kinetosomes does extend into this area from the anterior end of membranelle III and the individual kinetosomes of this row appear to be highly constant in position and number.

The four membranelles are interconnected by complex patterns of fibers that are revealed only in inside views of the oral apparatus. Prominent among these is a series of parallel fibers extending from membranelle II to membranelle III and what may be a continuation of this, a similar system extending from membrane III toward membranelle I (undulating membrane) and possibly connecting with the latter. These fibers appear to join with the membranelle kinetosomes. The appearance of these fibers suggests that they lie relatively deep below the oral pellicle. A second system of short, irregularly arranged fibers occupies a more superficial position. Neither of these systems has been studied in detail and they must be examined more thoroughly before an accurate description can be given.

Oral and kinetodesmal fibrils compared

A comparison of the oral fibers (Figs. 5, 6), especially the fan fibers from membranelle I, with the kinetodesmal fibrils (Figs. 1, 2, 3, 4) of the body ciliature im-

mediately reveals that the two types of fibers are quite different in structure. The kinetodesmal fibrils are short and rather uniform in length whereas the oral fibers appear to vary in length from membranelle to membranelle. The fan fibers in particular are very long. Moreover, the construction of the fibers from the two regions is strikingly different. The kinetodesmal fibrils have the marked periodic organization, clearly revealed in Figure 3. No trace of this periodicity appears in any of the figures of the oral fibers. These figures include fibers that are shadowed along, and at right angles to, the fiber axis. Some figures include both kinetodesmal fibrils with clean-cut periodicity and oral fibers lacking such organization. If the oral and kinetodesmal fibers are homologous structures, as would appear to be the case considering the currently accepted view of stomatogenesis, then these differences in organization must reflect specific differences in a single morphogenetic machine. Since this machine must operate fairly close to the macromolecular level, it and its products warrant special attention and more exhaustive study.

Discussion

This study provides new information of two sorts. The first and more significant of these is confirmatory information of a general nature, the second concerns the specific, detailed morphology of *Tetrahymena*.

Confirmatory

The observations reported here confirm the major contribution of the first study in this series. This contribution concerns the structure of the kineties of ciliates. These are now found to be built up of discrete units in *Tetrahymena* as well as in *Paramecium*. Each kinety unit consists of a cilium, kinetosome and a short tapering kinetodesmal fibril. The units are associated by overlap of the kinetodesmal fibrils to form the kineties and they are polarized toward the anterior of the animal in this association. The confirmation of this basic structure in *Tetrahymena* lends some support to the view that this construction may be widespread if not universal among ciliates.

This basic kinety structure has certain interesting implications for the physiology of conduction and coordination and for morphogenesis in ciliates. These aspects have been discussed at some length in the first paper of this series (Metz, Pitelka and Westfall, 1953) and they need not be repeated in detail here. With regard to conduction it is sufficient to emphasize that an acetylcholine-acetylcholine esterase system appears to be involved in the locomotion of *Tetrahymena* (Seaman, 1951; Seaman and Houlihan, 1951). This may operate in "synaptic" transmission from the kinetodesmal fibril of one kinety unit to that of the next unit (Metz, Pitelka and Westfall, 1953). The operation of such a mechanism is more readily visualized in *Tetrahymena* than in *Paramecium* because of the relatively simpler construction in the former organism. In *Tetrahymena* the overlapping kinetodesmal fibrils are so short that any single fibril contacts only the fibrils of the two units immediately adjacent to it (one anterior, the other posterior, to the given unit). Consequently, any coordinating impulse would necessarily pass stepwise from one kinetodesmal fibril to the next adjacent fibril. In *Paramecium* the kinetodesmal fibrils are relatively much longer, so much longer that they form bundles up to five fibrils in cross section.

Orderly transmission here would seem to require some selective agency (Metz, Pitelka and Westfall, 1953).

Because of its relative simplicity the fibrillar system of *Tetrahymena* should be favorable material for morphogenetic studies at the electron microscope level. It should not be difficult to obtain division stages showing multiplication of the kinety units. Even stages in stomatogenesis are not beyond expectation. But until such preparations are obtained, discussion of morphogenesis beyond that already given (Metz, Pitelka and Westfall, 1953) is of no great value.

Specific morphology

The morphology of *Tetrahymena* and related organisms has been studied extensively by a number of investigators using light optics combined with silver staining. Furgason (1940) and Corliss (1952a, 1953) have reviewed this work thoroughly and their accounts will serve as the basis for the following discussion. The electron optical preparations clarify to some extent the confusion that exists regarding the detailed anatomy of the "ciliary meridians" and certain aspects of the oral morphology in *Tetrahymena*. These particular problems will be considered here.

The ciliary meridians, as revealed by light optics and silver impregnation, are complex and somewhat variable structures. The most constant features include the "primary meridians" connecting the ciliary bases in longitudinal rows and "secondary meridians" running parallel to and approximately midway between the primary meridians. In some preparations even a third or tertiary meridional structure is described. Aside from these meridians transverse structures are frequently observed which extend on the left from the primary meridian to the vicinity of the secondary meridian. The fact that the appearance of these structures varies depending upon the extent of silver impregnation, has led some investigators to the view that many of the described structures are artifacts (*e.g.*, von Gelei, 1935). These structures may be "artifacts" in the sense that they do not exist in the living animal as actual fibers; but there must certainly be some specific, physical basis for them.

With the detailed picture of the kineties and associated pellicle now available from the electron photomicrographs the physical basis for certain of the silver structures may be discussed with some confidence. The "primary meridians," at least in heavily impregnated specimens, lie in the region of the overlapping kinetodesmal fibrils. The silver probably precipitates around, over, or in these fibers to give the effect observed in heavily impregnated material. In lightly treated preparations the meridians assume a lateral loop effect to the left between adjacent kinetosomes. The known structure of the system does not readily account for this phenomenon; nor does it account for the lateral extensions from the primary meridians in heavily impregnated material. However, in over-fixed animals prepared for electron microscopy (actually quite lightly fixed), cytoplasmic material appears to precipitate preferentially over the kineties and to extend as lateral projections in a more or less regular fashion (see Methods section). It is possible that such material serves as sites for silver precipitation.

The chains of ring-like thickenings in the pellicle readily account for the "secondary meridians" of light microscopy, assuming that some silver precipitates in the region of these structures. The longitudinally striated area of the pellicle lies to the right of the kineties and apparently remains rather free of silver, although a fine "fiber" is sometimes observed in this region with light optics.

The above account offers explanations for most of the meridional structures described in silver impregnated material. However, it should be recalled that the subpellicular cytoplasm was removed by the method of preparation for electron microscopy so there is ample room for further speculation. Indeed there appear to be many possible explanations for some of the structures observed in silver preparations and it is unlikely that exhausting them here would serve a useful purpose.

The electron photomicrographs of the oral structures reveal the wealth of detail described earlier in this paper. The relation of this detail to the interpretation of light optical studies should be obvious. However, there are two points that deserve further mention.

One of these is the margin of the oral apparatus. This is delimited on the right and part of the posterior edge by the first or "undulating" membranelle and on the left anterior margin by the second membranelle. Elsewhere the margins are free of membranelle structure. In these free regions the marginal pellicle may be somewhat thickened, but it appears more likely that it is supported by the underlying fiber network. Indeed it is likely that the entire oral apparatus is held together in these fixed preparations by the complex, interconnecting fiber system. Whether or not this fiber system serves a supportive function in the living animal is problematical. Nothing suggesting the circumoral ring of light optics appeared in the electron photomicrographs.

The second point of particular interest concerns the broad fan of fibers associated with the first or undulating membranelle. This structure has not been revealed in detail by light optical studies, but the rods, ribs (Corliss, 1953) and "zone intermédiaire" (Fauré-Fremiet, 1948b) that appear in silver preparations certainly represent fragments of the fan fibers. Furthermore, there is no longer any excuse for confusing the individual fibers of the fan with cilia.

Favorable preparations of the pre-oral region have not yet been obtained. The inferior ones that have been examined fail to show the "intermeridional connecting fibers" of light microscopy. Undoubtedly some basis for these in the form of fibers or pellicular sculpturing as well as other interesting morphological details will reward further investigation.

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Summary

1. Macronuclei, mitochondria, fragments of the pellicle and the oral apparatus of *Tetrahymena* may be isolated for electron microscopy by sonic dissection of osmium tetroxide fixed material. The fine morphology of the pellicle, the attached fibrillar structures and the oral apparatus are described.

2. The pellicle consists of a thin membrane which is sculptured to give a striated area immediately to the right of, and parallel to, each kinety, and two longitudinal ring-like thickenings, one at the level of and the other midway between the kineties.

3. The kineties of *Tetrahymena,* like those of *Paramecium,* are compound structures built up of discrete units. Each unit consists of three parts:

(a) The cilium which passes through a ring-shaped thickening in the pellicle.
(b) The ciliary basal body or kinetosome. This structure consists of two parts separated by a constriction.
(c) A short, tapering, kinetodesmal fibril which arises from the kinetosome.

These units of kinety structure are associated by their kinetodesmal fibrils. These fibrils overlap in shingle-like fashion to form the kinetodesma of the light microscopist. The individual kinetodesmal fibrils are highly polarized in this association. They all taper toward the anterior end of the animal. This mode of organization has now been demonstrated clearly in two ciliates, *Tetrahymena* and *Paramecium.* It will not be surprising if it is found to occur generally in ciliates.

4. The detailed structure of the oral apparatus, including the bases of the four membranelles and certain of their fibers, is revealed with new clarity. Prominent among the latter is a large fan-shaped group of fibers which arises from the first or undulating membranelle and probably extends deep into the interior of the animal.

5. The physical basis for some of the structures revealed to the light microscopist by silver impregnation is discussed.

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